Appendix B

Evaluation of the 304(a) Criteria for Cyanide to Determine Whether Threatened and Endangered Species are Likely to be Adversely Affect by Exposure at the CMC or CCC

Table 1 of the Cyanide Biological Opinion includes those listed species that are likely to be adversely affected by exposure to cyanide at the acute (CMC) and/or chronic (CCC) criterion. This determination was based on a comparison of the estimated sensitivity of individual species with the CMC and CCC.

The sensitivity of listed species to acute exposures was estimated by the Acute Assessment Effects Concentration or Acute EC_A. The Acute EC_A represents *the highest* concentration of cyanide where listed species are not likely to be adversely effected (see Appendix B1 for details). The Acute EC_A for each species was calculated by dividing the species LC_{50} (concentration of a toxicant that causes mortality in 50 percent of the exposed organisms) by a lethality threshold adjustment factor (LTAF). The LTAF was used to attenuate the concentration that causes 50 percent effect down to a concentration where adverse effects are not likely. The resulting Acute EC_A was then compared with the CMC (22 ug CN /L). If the Acute EC_A was lower than the CMC, the species was considered likely to be adversely affected. If the Acute EC_A was higher than the CMC, the species was considered not likely to be adversely affected.

Most listed species have not been tested for their sensitivity to cyanide; for those species LC_{50}s were estimated using data for surrogate species/taxa. Two methods were used to estimate LC_{50}s for listed species, ICE (Interspecies Correlation Estimates) and SSD (Species Sensitivity Distribution).

ICE method: According to the Draft BE Methods Manual (EPA 2006), Interspecies Correlation Estimates (ICEs) are based on regression analyses of LC_{50}s measured for a listed species to LC_{50}s measured for the same chemicals for commonly used surrogate species (based on a minimum of five tested chemicals) and the method is known to be reliable only through the family level. If the surrogate species has been tested for the chemical of interest, but the listed species of interest has not, such relationships are used to estimate the LC_{50} for the chemical and species of interest. When there is no ICE for the listed species, an ICE for its genus or family may be used. These higher taxa ICEs are derived the same as for individual listed species, except that each genus or family must be represented by at least two species. Due to the uncertainty in such correlations, the LC_{50} estimate used to calculate an acute EC_A is the lower bound of the 95% confidence interval of the ICE. Example estimates of the ICE model are provided in Appendix F of the Draft BE Methods Manual (EPA 2006).

SSD method: If several surrogate species within the same taxonomic unit as the species of interest have been the subject of acute toxicity tests, they can be used to estimate the species sensitivity distribution (SSD) of that taxonomic unit, and thus define possible LC_{50}s for the listed species of interest. To provide confidence in protecting the listed species, the 5^{th} percentile in this distribution will be used in Acute EC_A calculations. The method for estimating the 5^{th} percentile is described in Appendix G of the Draft BE Methods Manual (EPA 2006). For this SSD analysis to be conducted, there should be at least four tested species within the taxonomic unit being analyzed, and the tested species should include at least two taxa from the next lower taxonomic level (e.g., an analysis for a family should include data for at least two genera within that family). If only one lower taxon is represented and that taxon is considered a good representation of the listed species, then the SSD based on the one taxon can be accepted. The SSD method can be used through the class level (EPA 2006).

The prioritization scheme for using measured or modeled LC_{50} values for calculating Acute EC_As is described in Figure 2 of the Draft BE Methods Manual (EPA 2006). In general, measured LC_{50} values for listed species are preferred over modeled estimates and modeled estimates using the closest related taxonomic grouping are preferred over estimates based on more distantly related taxa.

The sensitivity of listed species to chronic exposures was estimated by the Chronic Assessment Effects Concentration or Chronic EC_A. The Chronic EC_A represents *the highest* concentration of cyanide where listed species are not likely to be adversely effected (see taxon-specific sections for details). Chronic EC_As were estimated using measured values if acceptable chronic toxicity data for the listed species were available. If such data were not available Chronic EC_As were calculated by dividing the listed species LC_{50} by the acute to chronic ratio (ACR). The ACRs were calculated using measured acute and chronic toxicity data for surrogate species (see taxon-specific sections for details). The resulting Chronic EC_A was then compared with the CCC (5.2 ug CN /L). If the Chronic EC_A was lower than the CCC, the species was considered likely to be adversely affected. If the Chronic EC_A was higher than the CCC, the species was considered not likely to be adversely affected.

Because cyanide does not tend to bioaccumulate, the sensitivity of aquatic listed species to direct cyanide toxicity was based on water-born exposure, as estimated by the Acute and Chronic EC_As. Listed aquatic taxa included fish, amphibians, freshwater mussels, and other aquatic invertebrates. The toxicity of cyanide on listed aquatic-dependent birds and mammal was evaluated based on their dietary exposure through ingestion of cyanide contained in prey.

Fish: Listed fish that were considered in this analysis appear in Table B1. Acute EC_As were calculated by dividing the species LC_{50} by an LTAF of 1.14 for salmonids and an LTAF 1.21 for all other fishes (see Appendix B1 for information on LTAF derivation). LC_{50} values were estimated according the prioritization scheme describe previously. The surrogate taxa and estimation method for each species are identified in Table B1. ICE estimates were based on ICE models listed in Appendix D of the Cyanide BO. LC_{50} values for surrogate species used to generate ICE estimates were from Table 1 of the Cyanide BE (EPA 2007). SSD-based estimates were from Table 2 of the Cyanide BE (EPA 2007). Chronic EC_As were calculated by dividing the listed species LC_{50} by 23.22; the ACR calculated for fish (see Appendix B2 for information on ACR derivation).

For species where the Acute EC_A was less than the CMC and/or the Chronic EC_A was less than the CCC a likely to adversely affect determination (LAA) was made. A not likely to adversely affect determination (NLAA) was made for species where the Acute EC_A was greater than the CMC and the Chronic EC_A were greater than the CCC.

Effects on listed fishes due to the toxicity of cyanide to prey items was assessed by EPA in the Cyanide BE (EPA 2007). Based on that analysis it appears that the greatest threat to affected fish is from direct toxicity.

For a detailed description of cyanide toxicity to fish, including lethal and sublethal effects, see the following sections in the Draft Biological Opinion; *Acute Toxicity to Fish* and *Chronic Toxicity to Fish*.

Table B1. Listed fish species that were evaluated for their sensitivity to cyanide relative to the acute (CMC) and chronic (CCC) cyanide criteria. For species where the Acute EC_A was less than the CMC (22.4 ug CN/L) and/or the Chronic EC_A were less than the CCC (5.2 ug CN/L) a likely to adversely affect determination (LAA) was made. A not likely to adversely affect determination (NLAA) was made for species where the Acute EC_A was greater than the CMC and the Chronic EC_A were greater than the CCC.

Listed Species		Order/Family	Surrogate Taxa	LC_{50} (ug CN/L)	Acute EC_A (ug CN/L)	Chronic EC_A (ug CN/L)	
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	Acipenseriformes Acipenseridae (sturgeon)	Actinopterygii (class)	66.50[1]	54.96	2.86	LAA
Kootenai River white sturgeon	*Acipenser transmontanus*						
Pallid sturgeon	*Scaphirhynchus albus*						
Alabama sturgeon	*Scaphirhynchus suttkusi*						
Waccamaw silverside	*Menidia extensa*	Atheriniformes Atherinopsidae					
Modoc sucker	*Catostomus micorps*	Cypriniformes Catosdomidae (suckers)	Cypriniformes (order)	84.55[1]	69.88	3.64	LAA
Santa Anna sucker	*Catostomus santaanae*						
Warner sucker	*Catostomus warnerensis*						
Shortnose sucker	*Chasmistes brevirostris*						
Cui ui	*Chasmistes cujus*						
June sucker	*Chasmistes liorus*						
Lost River sucker	*Deltistes luxatus*						
Razorback sucker	*Xyrauchen texanus*		*Xyrauchen texanus*	83.8[2]	69.26	3.61	LAA
Spotfin chub	*Cyprinella monacha*	Cypriniformes Cyprinidae	*Cyprinella monacha*	36.7[2]	30.33	1.58	LAA
Blue shiner	*Cyprinella caerulea*		Cyprinidae (family)	101.7[2]	84.05	4.38	LAA
Beautiful shiner	*Cyprinella formosa*						
Devils River minnow	*Dionda diaboli*						
Slender chub	*Erimystax cahni*						
Mohave tui chub	*Gila bicolor mohavensis*						
Owens tui chub	*Gila bicolor snyderi*						
Hutton tui chub	*Gila bicolor* ssp.						

Borax Lake chub	*Gila boraxobius*						
Humpback chub	*Gila cypha*						
Sonora chub	*Gila ditaenia*						
Gila chub	*Gila intermedia*						
Yaqui chub	*Gila purpurea*						
Pahranagat roundtail chub	*Gila robusta jordani*						
Virgin River chub	*Gila robusta seminude*						
Rio Grand silvery minnow	*Hybognathus amarus*						
Big Spring spinedace	*Lepidomeda mollispinis pratensis*						
Little Colorado spinedace	*Lepidomeda vittata*						
Spikedace	*Meda fulgida*						
Moapa dace	*Moapa coriacea*						
Palezone shiner	*Notropis albizonatus*						
Cahaba shiner	*Notropis cahabae*						
Arkansas River shiner	*Notropis girardi*						
Pecos bluntnose shiner	*Notropis simus pecosensis*						
Topeka shiner	*Notropis Topeka*						
Oregon chub	*Oregonichthys crameri*						
Blackside dace	*Phoxinus cumberlandensis*						
Woundfn	*Plagopterus agrentissimus*						
Ash Meadows speckled dace	*Rhinichthys osculus nevadensis*						
Kendall Warm Springs dace	*Rhinichthys osculus thermalis*						
Foskett speckled dace	*Rhinichthys osculus* ssp.						
Loach minnow	*Tiaroga cobitis*						
Bonytail chub	*Gila elegans*		*Gila elegans*	50.9[2]	42.07	2.19	LAA
Cape Fear shiner	*Notropis mekistocholas*		*Notropis mekistocholas*	48.51[2]	40.09	2.09	LAA
Colorado pikeminnow	*Ptychocheilus lucis*		*Ptychocheilus lucis*	43.45[2]	35.91	1.87	LAA
Leon springs pupfish	*Cyprinodon bovinus*	Cyprinodontiformes Cyprinodontidae	*Cyprinodon* (genus)	127.7[2]	105.54	5.50	NLAA
Comanche Springs pupfish	*Cyprinodon elegans*						
Desert pupfish	*Cyprinodon macularius*						
Ash Meadows Amargosa pupfish	*Cyprinodon nevadensis mionectes*						
Warm springs pupfish	*Cyprinodon nevadensis pectoralis*						
Owens pupfish	*Cyprinodon radiosus*						
White River springfish	*Crenichthys baileyi baileyi*	Cyprinodontiformes Goodeidae	Actinopterygii (class**)**	66.50[1]	54.96	2.86	LAA
Hiko White River springfish	*Crenichthys baileyi grandis*						
Railroad Valley springfish	*Crenichthys nevadae*						
Big Bend gambusia	*Gambusia gaigei*	Cyprinodontiformes Poeciliidae					
San Marcos gambusia	*Gambusia georgei*						
Clear Creek gambusia	*Gambusia heterochir*						

Pecos gambusia	*Gambusia nobilis*						
Gila topminnow	*Poeciliopsis occidentalis occidentalis*						
Yaqui topminnow	*Poeciliopsis occidentalis sonoriensis*						
Unarmoned threespine stickleback	*Gasterosteus aculeatus williamsoni*	Gasterosteiformes Gasterosteidae					
Delta smelt	*Hypomesus transpacificus*	Osmeriformes Osmeridae					
Tidewater goby	*Eucyclogobius newberryi*	Perciformes Gobiidae	Perciformes (order)	90.80[1]	75.04	3.91	LAA
Slackwater darter	*Etheostoma boschungi*	Perciformes Percidae	*Etheostoma* (genus)	40.01[2]	33.07	1.72	LAA
Vermilion darter	*Etheostoma chermocki*						
Relict darter	*Etheostoma chienense*						
Etowah darter	*Etheostoma etowahae*						
Niangua darter	*Etheostoma nianguae*						
Watercress darter	*Etheostoma nuchale*						
Okaloosa darter	*Etheostoma okaloosae*						
Duskytail darter	*Etheostoma percnurum*						
Bayou darter	*Etheostoma rubrum*						
Cherokee darter	*Etheostoma scotti*						
Maryland darter	*Etheostoma sellare*						
Bluemask darter	*Etheostoma sp.*						
Boulder darter	*Etheostoma wapiti*						
Fountain darter	*Etheostoma fonticola*		*Etheostoma fonticola* (species)	21.53[2]	**17.2**	0.93	LAA
Amber darter	*Percina antesella*		Percidae (family)	42.31[2]	34.97	1.82	LAA
Goldline darter	*Percina aurolineata*						
Conasauga logperch	*Percina jenkinsi*						
Leopard darter	*Percina pantherina*						
Roanoke logperch	*Percina rex*						
Snail darter	*Percina tanasi*						
Ozark cavefish	*Amblyopsis rosae*	Percopsiformes Amblyopsidae	Actinopterygii (class**)**	66.50[1]	54.96	2.86	LAA
Alabama cavefish	*Speoplatyrhinus poulsoni*						
Little Kern golden trout	*Oncorhynchus aguabonita whitei*	Salmoniformes Salmonidae	*Oncorhynchus* (genus)	47.02[2]	41.24	2.02	LAA
Paiute cutthroat trout	*Oncorhynchus clarki seleniris*						
Greenback cutthroat trout	*Oncorhynchus clarki stomias*						
Gila trout	*Oncorhynchus gilae*						
Apache trout	*Oncorhynchus apache*		*Oncorhynchus apache* (species)	16.51[2]	**14.47**	0.71	LAA
Lahontan cutthroat trout	*Oncorhynchus clarki henshawi*		*Oncorhynchus clarki henshawi* (species)	22.83[2]	**20.00**	0.98	LAA
Atlantic salmon	*Salmo salar*		*Salmo salar* (species)	90[3]	78.95	3.87	LAA
Bull trout	*Salvelinus confluentus*		*Salvelinus* (genus)	15.72[2]	**13.77**	0.68	LAA
Pygmy sculpin	*Cottus paulus*	Scorpaeniformes	Actinopterygii	66.50[1]	54.96	2.86	LAA

		Cottidae	(class)				
Yaqui catfish	*Ictalurus pricei*	Siluriformes Ictaluridae	Ictaluridae (family)	182.8[2]	151.07	7.87	NLAA
Smoky madtom	*Noturus baileyi*						
Yellow madtom	*Noturus flavipinnis*						
Neosho madtom	*Noturus placidus*						
Pygmy madtom	*Noturus stanauli*						
Scioto madtom	*Noturus trautmani*						

[1] LC_{50} based on 5^{th} percentile estimate from species sensitivity distribution (SSD), Table 2 – Cyanide BE (EPA 2007).
[2] LC_{50} estimate based on lower bound of the 95% CI from ICE model (Appendix C).
[3] LC_{50} based on measured value from Cyanide BE (Table 1).

Freshwater Mussels: Like all taxa, the sensitivity of mussels to contaminants is variable. Laboratory toxicity tests have found mussels to be relatively insensitive to certain solvents and pesticides, and amongst the most sensitive aquatic organisms to copper and ammonium (Augspurger et al 2007). Therefore, the sensitivity of mussels to particular contaminants is best assessed on a chemical-specific basis. In the case of cyanide, the limited data set available for direct effects to mussels from cyanide exposure did not allow for its use in assessment of effects of criteria concentrations to freshwater unionoid mussels. Of the data available for the class Bivalvia, none of the three species for which data exist share a common taxonomy with freshwater mussels below the class level, and two of the species (common blue mussel, *Mytilus edulis,* and arcid blood clam, *Scapharca inaequivalvis*) are marine organisms. For the blue mussel, a 96-hour LC_{50} of 36 mg/L was estimated, and concentrations required to affect filtration rate by 50% ranged between 0.25 – 0.33 mg/l (Abel 1974). Though the described test was not robust enough to detect changes below 50%, the author speculates that populations exposed to lower concentrations in the field may suffer ecological impairment. In a study with fingernail clams (*Musculium transversum*), filtration rates were reduced by 50% at sodium cyanide concentrations of approximately 1 – 5 mg/l (Sparks and Dillon 1998). The arcid blood clam experienced 50% mortality in 11.2 days when exposed to 26 mg/L cyanide (DeZwaan, et al. 1993). The arcid mussel differs significantly from most bivalves in that it produces hemoglobin-containing erythrocytes that allow for greater efficiency in extracting oxygen from water.

In the absence of applicable data for unionid mussels, direct effects were assessed by estimating LC_{50} values for the genus *Lampsilis* and the family Unionidae using EPA's ICE model, with rainbow trout (*Oncoryhnchus mykiss)* as a surrogate. Acute EC_A values were calculated by dividing the LC_{50} by the cyanide-specific 1.21 factor derived by the Service. Chronic EC_A values were derived by dividing the estimated LC_{50} by the invertebrate ACR, 8.889. All values fell above both acute (22.4 ug/L) and chronic (5.2 ug/L) criteria values, and therefore, no direct effects to mussels are anticipated at criteria concentrations:

	Estimated LC_{50} (ug/L)	Acute EC_A (ug/L)	Chronic EC_A (ug/L)
Lampsilis	149.13	123.24	16.78
Unionidae	114.73	94.81	12.91

Exposure of freshwater mussels to waterborne contaminants differs according to life history and stage of development.

Adult mussels are long-lived organisms that burrow in the sediments of rivers, streams, and lakes and obtain food either via suspension feeding, or deposit feeding. Thus, the primary routes of exposure are surface water, sediment, pore water, and diet, consisting of detritus, zooplankton, bacteria and algae. Adults can be exposed to contaminants while either partially or completely burrowed in the sediment (Cope et al 2008). Though adults have typically been thought to be less sensitive to contaminants than early life-stages (glochidia and juveniles), recent studies have demonstrated that typical acute laboratory tests may not be applicable in estimating the effects of chronic low-level exposure. Effects to adults can be reduced in acute exposures by the ability to detect and avoid a toxicant through valve closure, a mechanism that cannot be sustained for prolonged periods. Mussels subject to chronic exposures have exhibited accumulation of toxicants and adverse effects that have not previously been demonstrated (Cope et al 2008).

Glochidia experience contaminant exposure from surface waters after release from adults and prior to attachment on host fish, lasting from days to weeks. Once fully encysted on a host, glochidia are likely to be protected from waterborne contaminants, but feed on host fish tissue for weeks to months during transformation (Cope et al 2008). There has been no research on this potential avenue of exposure. After transformation, juvenile mussels typically burrow for 2-4 years, consuming fine particulate organic matter such as detritus, bacteria, and algae through deposit and pedal feeding. Toxicity tests on glochidia and newly transformed juveniles tend to yield similar results, though recent studies assessing sediment-based exposure revealed LC_{50}'s 2-3 times lower than water-only exposures for the same contaminant, indicating possible increased sensitivity of the juvenile stage due to this route (Cope et al 2008).

<u>Summary of exposure routes for mussels:</u>

Life Stage:	*Exposure Route:*
Adult	Surface water, pore water, sediment, diet
Glochidia (brooded and free)	Surface water
Glochidia (encysted)	Surface water, host fish tissue
Juvenile	Surface water, pore water, sediment, diet

(adapted from Cope et al 2008)

For this analysis, we assume waterborne exposure to cyanide to be the primary form of toxicity for all life stages. Though cyanide sediment toxicity testing has not occurred for mussels, cyanide ions are not significantly adsorbed onto soils, and will likely leach into surrounding pore water. In addition, no data exist to assess cyanide exposure to mussels via food items or fish tissue. However, since biomagnification of cyanide has not been reported, presumably due to rapid biotransformation and detoxification in the body, circulating concentrations in tissues of host fish or prey items are likely to be low.

Other Aquatic Invertebrates: Listed invertebrates other than freshwater mussels that were considered in this analysis appear in Table B2. Direct effects to invertebrates were assessed by estimating LC_{50} values using EPA's ICE model for the genus *Gammarus* (with *Daphnia magna* as a surrogate), or using species sensitivity distributions (SSD) for the class Malacostraca, class Insecta, and order Basommatophora. For species where ICE models or SSDs through the class level are unavailable, best professional judgment was exercised to estimate effect levels from closely related species, as described in Table B2. Acute EC_A values were calculated by dividing the LC_{50} by the cyanide-specific 1.21 factor derived by the Service. Chronic EC_A values were derived by using measured NOECs or by dividing the estimated LC_{50} by the invertebrate ACR of 8.889 where a NOEC was not available. With the exception of the chronic EC_A values for the Illinois cave amphipod and Noel's amphipod, all other EC_A values fell above both acute (22.4 ug/L) and chronic (5.2 ug/L) criteria values, and therefore, no direct effects to mussels are anticipated at criteria concentrations. Chronic effects data are not sufficiently robust to evaluate EC_{10} levels for invertebrates.

Table B2. Listed invertebrate species (other than freshwater mussels) that were evaluated for their sensitivity to cyanide relative to the acute (CMC) and chronic (CCC) cyanide criteria. For species where the Acute EC_A was less than the CMC (22 ug CN/L) and/or the Chronic EC_A were less than the CCC (5.2 ug CN/L) a likely to adversely affect determination (LAA) was made. A not likely to adversely affect determination (NLAA) was made for species where the Acute EC_A was greater than the CMC and the Chronic EC_A were greater than the CCC.

Listed Species		Order/Family	Surrogate Taxa	LC_{50}	Acute EC_A	Chronic EC_A	
Illinois cave amphipod	*Gammarus acherondytes*	Amphipoda Cambaridae	*Gammarus* (genus)	29.63[I]	24.49	3.33	LAA
Noel's Amphipod	*Gammarus desperatus*			29.63[I]	24.49	3.33	LAA
Hay's Spring amphipod	*Stygobromus hayi*	Amphipoda Crangonyctidae	Malacostraca (class)	66.57[S]	55.02	8.53	NLAA
Peck's cave amphipod	*Stygobromus pecki*			66.57[S]	55.02	8.53	NLAA
Kauai cave amphipod	*Spelaeorchestia koloana*	Amphipoda Talitridae		66.57[S]	55.02	8.53	NLAA
Conservancy fairy shrimp	*Branchinecta conservatio*	Anostraca Branchinectidae	Branchiopoda (class)	95.55[1]	78.97	10.75	NLAA
Longhorn fairy shrimp	*Branchinecta longiantenna*			95.55[1]	78.97	10.75	NLAA
San Diego fairy shrimp	*Branchinecta sandiegoensis*			95.55[1]	78.97	10.75	NLAA
Vernal pool fairy shrimp	*Branchinecta lynchi*			95.55[1]	78.97	10.75	NLAA
Riverside fairy shrimp	*Streptocephalus wootoni*	Anostraca Streptocephalidae		95.55[1]	78.97	10.75	NLAA
White abalone	*Haliotis sorenseni*	Archaeogastropoda Haliotidae	*Haliotis* (genus)	1012[2]	836.4	424.5	NLAA
Banbury springs limpet	*Lanx sp.*	Basommatophora Lancidae	Basommatophora (order)	247.4[S]	204.46	27.84	NLAA
Comal springs dryopid beetle	*Stygoparnus comalensis*	Coleoptera Dryopidae	Insecta (class)	216.2[S]	178.67	24.34	NLAA
Comal Springs riffle beetle	*Heterelmis comalensis*	Coleoptera Elmidae		216.2[S]	178.67	24.34	NLAA
Hungerford's crawling water beetle	*Brychius hungerfordi (larvae)*	Coleoptera Haliplidae		216.2[S]	178.67	24.34	NLAA
Alabama cave	*Palaemonias*	Decapoda Atyidae	Malacostraca	66.57[S]	55.02	8.53	NLAA

shrimp	*alabamae*		(class)				
California freshwater shrimp	*Syncaris pacifica*			66.57[S]	55.02	8.53	NLAA
Kentucky cave shrimp	*Palaemonias ganteri*			66.57[S]	55.02	8.53	NLAA
Nashville crayfish	*Orconectes shoupi*	Decapoda Cambaridae		66.57[S]	55.02	8.53	NLAA
Shasta crayfish	*Pacifastacus fortis*			66.57[S]	55.02	8.53	NLAA
Squirrel chimney cave shrimp	*Palaemonetes cummingi*	Decapoda Palaemonidae		66.57[S]	55.02	8.53	NLAA
Lee County cave isopod	*Lirceus usdagalun*	Isopoda Cirolanidae		66.57[S]	55.02	8.53	NLAA
Madison cave isopod	*Antrolana lira*			66.57[S]	55.02	8.53	NLAA
Socorro isopod	*Thermosphae roma thermophilus*	Isopoda Sphaeromatidae		66.57[S]	55.02	8.53	NLAA
Vernal pool tadpole shrimp	*Lepidurus packardi*	Notostraca Triopsidae	Branchiopoda (class)	95.55[1]	78.97	10.75	NLAA
Hine's emerald dragonfly	*Somatochlora hineana (Larvae)*	Odonata Corduliidae	Insecta (class)	216.2 [S]	178.67	24.34	NLAA

[S] LC_{50} based on 5[th] percentile estimate from species sensitivity distribution (SSD), Table 2 – Cyanide BE (EPA 2007).
[I] LC_{50} estimate based on lower bound of the 95% CI from ICE model (Appendix C).

1 Too few data exist to generate an SSD estimate for this species up through the class level. The mean LC_{50} and NOEC values for the most closely related species in Table 1 (*Daphnia sp.* within class Branchiopoda) range from 95.55 to 169 and 10.75 to 19.02 µg CN/L, respectively. The most conservative LC_{50} and NOEC values for *Daphnia sp.* (i.e., mean LC_{50} and NOEC for *D. pulex*) were used as surrogates for this species.

2 Too few data exist to generate an SSD estimate for this species up through the class level. The LC_{50} value for the most closely related species, *Haliotis varia* was used as a surrogate for this species, and the chronic $\mathbf{EC_A}$ derived using the saltwater invertebrate ACR of 2.384.

Amphibians: Our assessment of the sensitivity of listed amphibian species to cyanide was based on multiple lines of evidence. First, we evaluated the available information on cyanide-induced effects on amphibians. We then reviewed the approach EPA used in their Biological Evaluation to assess the sensitivity of listed amphibians to cyanide and the protectiveness of the cyanide criteria. Next, we examined additional toxicity information for amphibians, not used by EPA, and constructed regression models for predicting the acute sensitivities of amphibian genera to cyanide. Finally, we compared the predicted sensitivity of amphibians with that of rainbow trout; the most sensitive freshwater species (based on measured cyanide LC_{50}s) and the species that was used to set the acute and chronic cyanide criteria. Taken together, these data provided the basis for our effects determination.

The scientific literature for cyanide toxicity to amphibians is limited and somewhat dated. Early investigators studied the effects of cyanide on amphibian development. These experiments were generally focused on early embryogenesis including oviposited and fertilized egg morphogenesis and post gastrulation development. Repressive effects of cyanide on embryonic respiration and development were documented by several authors

(Spiegelman and Moog, 1943, Lovtrup and Pigon, 1958, Nakatsuji, 1974). Others used sub-lethal exposure concentrations of cyanide as a mechanism to arrest or retard development in order to test various hypotheses regarding metabolism or physiology (Spiegelman and Steinbach, 1945; Ornstein and Gregg, 1952). Although these historical studies are important for understanding the physiological actions of cyanide on amphibians, they do not provide the traditional quantitative measures of acute and chronic toxicity (i.e. LC_{50}s, NOECs, EC_xs) that have been used in water quality criteria development.

Because cyanide-specific toxicity data (LC_{50}s) for amphibians were not available, EPA based their effects analysis on the relative sensitivity of amphibians to other pollutants (EPA 2007). They examined the rank order of amphibian LC_{50}s for seven water pollutants using data sets from ambient water quality criteria documents (Table B3). The 7 data sets included LC_{50}s for 9 amphibian species (in total), although 4 of the data sets contained LC_{50}s for only 1 amphibian species and the other 3 data sets contained data for 2 species. So among these seven criteria documents, the amphibian class was represented by no more than one or two species at a time. With so few species used to characterize the sensitivity of an entire class there is considerable uncertainty as to whether the most sensitive amphibian species are adequately represented, especially considering the large interspecies variability in cyanide toxicity observed for other taxa (see acute effects section of BO). It seems highly unlikely that the amphibians species included in these data sets were among the most sensitive amphibians. Nevertheless, for two of the seven pollutants the single amphibian species in the data set ranked among the most sensitive species/genera in the multi-taxa data sets used to develop criteria. For the remaining five pollutants the GMAVs for amphibians ranged from the 26th percentile to the 100th percentile. Considering the low number of species used to represent amphibians in the analysis and the fact that amphibians were among the most sensitive species/genera for 28% of the pollutants examined we believe that there is a more than a discountable chance that some amphibian species may be highly sensitive to cyanide. Therefore, we do not believe these results alone support EPAs determination that the listed amphibian species are not likely to be adversely affected by cyanide at criteria concentrations.

To better understand how to interpret the results from EPAs analysis we extended our evaluation to include rainbow trout; a species frequently included in criteria development and often among the more sensitive species tested (Table B3). Using data for the same seven pollutants we found that the over all pattern of rankings for rainbow trout were much like those for amphibians, i.e. most near or above the median and two or three falling among the most sensitive species. However we know that in terms of cyanide, rainbow trout is the most sensitive freshwater species that has been tested, more sensitive than the 5th percentile estimated species (EPA 1985). (That is, rainbow trout fell in the "sensitive tail" of the species sensitivity distribution.) So, there is at least one example where the "ranking profile" (for these 7 pollutants) shared by amphibians and rainbow trout was associated with a species that was highly sensitive to cyanide. In addition, we found that for these seven pollutants amphibian species were more sensitive than rainbow trout 43% of the time (3 of 7). To further investigate the relative sensitivity of amphibians to other taxa we reviewed other references on amphibian toxicology.

Table B3. Rank and corresponding percentile of GMAVs (genus mean acute values) for amphibians and rainbow trout versus all aquatic taxa and chordates (fishes) only. Data for amphibians are from Appendix D of EPAs Cyanide Biological Evaluation (EPA 2007). Data for rainbow trout are from criteria documents (see footnotes).

Chemical	Amphibian Species	Amphibian GMAV Rank Vs. Other Taxa	Rainbow (GMAV) Rank vs. Other Taxa	Percentile (Amphibians)	Percentile (Rainbow trout)	Amphibians more (+) or less (-) sensitive than Rainbow trout
Atrazine	Bufo americanus	11 of 19	4 of 19[1]	0.58	0.21	-
Atrazine	Rana sp.	14 of 19		0.74		
Cadmium	Ambystoma gracile	29 of 57	4 of 57[2]	0.51	0.07	-
Cadmium	Xenopus laevis	33 of 57		0.58		
Diazinon	Rana clamitans	8 of 21	12 of 21[3]	0.26	0.57	+
Lindane	Pseudacris triseriata	22 of 23	10 of 23[4]	0.96	0.43	-
Lindane	Bufo woodhousei	23 of 23		1.00		
Nonylphenol	Bufo boreas	2 of 15	8 of 15[5]	0.13	0.53	+
Parathion	Pseudacris triseriata	23 of 31	25 of 31[5]	0.74	0.81	+
Pentachlorophenol	Rana satesbeiana	4[6] of 32	3 of 32[5]	0.13	0.09	-

[1] Draft aquatic life ambient water quality criteria for atrazine (EPA 2003)
[2] 2001 update of the aquatic life ambient water quality criteria for cadmium (EPA 2001)
[3] Aquatic life ambient water quality criteria for diazinon (EPA 2005)
[4] 1995 updates: water quality criteria documents for the protection of aquatic life in ambient water (EPA 1996)
[5] Aquatic life ambient water quality criteria for nonylphenol (EPA 2005)
[6] rank was changed from 5 to 4 based on GMAV ranks for pentachlorophenol (EPA 1996)

Birge at al (2003) performed a comparative toxicity analysis for 29 amphibian species in contrast to various species of fish. Amphibian testing included seven salamander species (family Ambystomidae) and 22 frog species (families Microhylidae, Hylidae, Ranidae, and Bufonidae). Though no toxicity testing was performed for cyanide, sufficient data was produced to generate comparisons between amphibians and fish for 34 inorganic compounds and 27 organic compounds. Comparisons include all amphibian test species for 50 of these 61 compounds. Although exposure times varied among species due to differences in hatching times, comparable stages of development (eggs, embryos, and early larvae) were included in all tests. Fish species included in this study for which sensitivity to cyanide is known are the rainbow trout (LC_{50} = 59.22ug/g), largemouth bass (101.7 ug/g) and fathead minnow (138.4 ug/g).

When compared to rainbow trout, LC_{50} values for amphibians were more sensitive 52% of the time for metals (N=203), 36% for organics (N=44), and 49% for all compounds combined (N=247). For largemouth bass, amphibians were more sensitive 83% of the time (N=182), 60% for organics (N=15), and 81% for all compounds (N=197). For fathead minnow, amphibians were more sensitive 89% of the time for metals (N=18), 63% for organics (N=24), and 74% for all compounds (N=42). The generally more sensitive species of Microhylidae and Hylidae were not available for toxicity testing for several organic compounds. For the 15 most sensitive amphibian species, LC_{50} values were below fish values (including species used above, plus channel catfish and goldfish) 74% of the time.

Bridges et al (2002), performed toxicity testing for five compounds on southern leopard frog (*Rana sphenocephala*) tadpoles and compared results with published values for the boreal toad (*Bufo boreas*), rainbow trout, fathead minnow, and bluegill. The two amphibian species showed the highest correlation of LC_{50} values for the rainbow trout. Correlations for the fathead minnow and bluegill were much weaker. The authors suggest that rainbow trout thus may be the most appropriate species for assessing toxicity to anuran tadpoles. However, the authors also argue that since amphibians are very tolerant to some chemicals, and very sensitive to others, individual toxicity testing is suggested rather than relying on surrogate species.

The comparative toxicity data sets from Birge et al. (2003) provided an opportunity to construct ICE-like regression models that could be used to estimated cyanide LC_{50}s for amphibians. Following EPA guidelines (EPA 2003), regression models were developed to estimate the sensitivity of two amphibian genera (Rana and Ambystoma) using rainbow trout as the surrogate species:

Predicted Taxon	Surrogate Species	LCI LC_{50} (ug/L)	MLE LC_{50} (ug/L)	UCI LC_{50} (ug/L)	Corr. Coeff. (r)	MSE	log-log a	log-log b	p	n	Chem.
Rana (genus)	Rainbow Trout	30.82	54.25	95.51	0.789	0.648	0.259	0.832	<0.001	84	32
Ambystoma (genus)	Rainbow Trout	60.56	120.61	639.63	0.638	0.415	0.966	0.629	<0.002	23	4

Estimated LC_{50}s for the two amphibian genera, Ambystoma (LC_{50} 60.56 ug CN/L) and Rana (LC_{50} 30.82 ug CN/L), are approximately equal to or less than the LC_{50} for rainbow trout (59 ug CN/L).

As previously mentioned, rainbow trout had the lowest measured cyanide LC_{50} of all fish species considered in the cyanide criteria document as well as the cyanide BE. Based on the method described in the *Fish* section of Appendix B, the chronic EC_A for rainbow trout would be 2.54 ug CN/L (i.e. 59 ug CN/L / 23.22) and the acute EC_A would be 51.75 ug CN/L (i.e. 59 ug CN/L / 1.14). Because the chronic EC_A is below 5.2 ug CN/L rainbow trout would likely be adversely affected by exposure to cyanide at the CCC. Thus, amphibian species that are estimated to be as sensitive or more sensitive to cyanide than rainbow trout are also likely to be adversely affected by exposure to cyanide at the chronic criterion.

Conclusions

Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that all 18 amphibian species/DPS's considered in this BO are likely to be adversely affected by exposure to cyanide at the chronic criterion (Table B4).

Table B4. Listed amphibian species that were evaluated for their sensitivity to cyanide relative the CCC (NLAA - not likely to be adversely affected; LAA - likely to be adversely affected).

Species Common Name	**Scientific Name**	**EPA BE**	**FWS Effects Determination**
Reticulated flatwoods salamander	*Ambystoma bishop*	May affect, NLAA	LAA
California tiger salamander	*Ambystoma californiese* Central California DPS	May affect, NLAA	LAA
California tiger salamander	*Ambystoma californiese* Santa Barbara County DPS	May affect, NLAA	LAA
California tiger salamander	*Ambystoma californiense* Sonoma County DPS	May affect, NLAA	LAA
Frosted Flatwoods Salamander	*Ambystoma cingulatum*	May affect, NLAA	LAA
Santa Cruz long-toed salamander	*Ambystoma macrodactylum croceum*	May affect, NLAA	LAA
Sonora Tiger salamander	*Ambystoma tigrinum stebbinsi*	May affect, NLAA	LAA
Wyoming Toad	*Bufo baxteri*	May affect, NLAA	LAA
Arroyo Toad	*Bufo californicus*	May affect, NLAA	LAA
Houston Toad	*Bufo houstonensis*	May affect, NLAA	LAA
Guajon	*Eleutherodactylus cooki*	Not addressed	LAA
San Marcos salamander	*Eurycea nana*	May affect, NLAA	LAA
Barton Springs salamander	*Eurycea sosorum*	May affect, NLAA	LAA
California red-legged frog	*Rana aurora draytonii*	May Affect, NLAA	LAA
Chiricahua Leopard Frog	*Rana chiricahuensis*	Not addressed	LAA

Mountain yellow-legged frog	*Rana muscosa*	Not addressed	LAA
Texas Blind Salamander	*Typhlomolge rathbuni*	May affect, NLAA	LAA

Aquatic-Dependent Species: The sensitivity of listed aquatic-dependent species to cyanide was assessed based on chronic exposure to cyanide via dietary aquatic food items. The Chronic Effects Concentration or Chronic EC_A was used to estimate sensitivity. The Chronic EC_A represents *the highest* concentration of cyanide where the effects on listed species are expected to be insignificant. The Chronic EC_A for each species was compared to the estimated cyanide concentration in food items. If the Chronic EC_A was lower than the estimated dietary concentration, the species was considered likely to be adversely affected. If the Chronic EC_A was higher than the estimated concentration in food items, the species was considered not likely to be adversely affected.

EPAs effects analysis for aquatic-dependent taxa in the Cyanide BE (EPA 2007) indicates that few chronic data sets were available for estimating chronic EC_As. They estimated the Chronic EC_A to be >2.40 mg/kg food based on a chronic study with Wistar rats. The appropriateness of using this single value to estimate the sensitivity of all taxa of aquatic dependent species, i.e. mammals, birds, reptiles, as well as some life stages of amphibians, insects and freshwater mussels is highly questionable. EPA estimated the concentration of cyanide in aquatic food items to be 0.0052 mg CN/kg; a factor of (>) 462 times less than the estimated Chronic EC_A. They also reported the acute toxicity (LD_{50}) for 13 vertebrate species (mammals and birds), which varied by a factor of 7 between most and least sensitive. Because cyanide does not tend to bioaccumulate and the estimated dietary concentration of cyanide is so far below the estimated chronic effects threshold (>462 times lower) we conclude that dietary ingestion should not result in adverse effects to listed mammals, birds, and reptiles.

Appendix B1

Recalculation of the Lethality Threshold Adjustment Factor (LTAF) for Fish

EPA's (2007) final Biological Evaluation (BE) identified 31 species of fish and 1 species of invertebrate for which the acute effects assessment concentrations (EC_As) were lower than the current acute (CMC) criterion for cyanide of 22.4 ug CN/L, as listed below (from EPA 2007:Table 4):

Species: common name	**Species: scientific name**	**BE Acute EC_a (ug CN/L)**
FISH:		
Amber Darter	*Percina antesella*	20.04 (ICE-Percidae)

Apache Trout	*Oncorhynchus apache*	9.08 (ICE-*O. apache*)
Bayou Darter	*Etheostoma rubrum*	18.93 (ICE-*Etheostoma*)
Bluemask Darter	*Etheostoma sp.*	18.93 (ICE-*Etheostoma*)
Boulder Darter	*Etheostoma wapiti*	18.93 (ICE-*Etheostoma*)
Bull Trout	*Salvelinus confluentus*	8.62 (ICE-*Salvelinus*)
Cherokee Darter	*Etheostoma scotti*	18.93 (ICE-*Etheostoma*)
Chinook Salmon	*Oncorhynchus tshawytscha*	16.26 (ICE-*O. tshawytscha*)
Chum Salmon	*Oncorhynchus keta*	21.41 (ICE-*Oncorhynchus*)
Coho Salmon	*Oncorhynchus kisutch*	15.51 (ICE-*O. kisutch*)
Conasauga logperch	*Percina jenkinsi*	20.04 (ICE-Percidae)
Duskytail Darter	*Etheostoma percnurum*	18.93 (ICE-*Etheostoma*)
Etowah Darter	*Etheostoma etowahae*	18.93 (ICE-*Etheostoma*)
Fountain Darter	*Etheostoma fonticola*	11.33 (ICE-*E. fonticola*)
Gila Trout	*Oncorhynchus gilae*	21.41 (ICE-*Oncorhynchus*)
Goldline Darter	*Percina aurolineata*	20.04 (ICE-Percidae)
Greenback Cutthroat Trout	*Oncorhynchus clarki stomias*	21.41 (ICE-*Oncorhynchus*)
Lahontan Cutthroat Trout	*Oncorhynchus clarki henshawi*	11.85 (ICE-*O. c. henshawi*)
Leopard Darter	*Percina pantherina*	20.04 (ICE-Percidae)
Little Kern Trout	*O. aguabonita whitei*	21.41 (ICE-*Oncorhynchus*)
Maryland Darter	*Etheostoma sellare*	18.93 (ICE-*Etheostoma*)
Niangua Darter	*Etheostoma nianguae*	18.93 (ICE-*Etheostoma*)
Okaloosa Darter	*Etheostoma okaloosae*	18.93 (ICE-*Etheostoma*)
Paiute Cutthroat Trout	*Oncorhynchus clarki seleniris*	21.41 (ICE-*Oncorhynchus*)
Relict Darter	*Etheostoma chienense*	18.93 (ICE-*Etheostoma*)
Roanoke Logperch	*Percina rex*	20.04 (ICE-Percidae)
Slackwater Darter	*Etheostoma boschungi*	18.93 (ICE-*Etheostoma*)
Snail Darter	Percina tanasi	20.04 (ICE-Percidae)
Sockeye Salmon	*Oncorhynchus nerka*	21.41 (ICE-*Oncorhynchus*)
Spotfin Chub	*Cyprinella monacha*	18.50 (ICE-*C. monacha*)
Watercress Snail Darter	*Etheostoma nuchale*	18.93 (ICE-*Etheostoma*)
INVERTEBRATES:		
Illinois Cave Amphipod	*Gammarus acherondytes*	15.33 (ICE-*Gammarus*)

In addition, subsequent to the submission of EPA's (2007) final BE, the Vermilion Darter (*Etheostoma chermocki*) was added to the species list for this consultation and, along with most other *Etheostoma* darters, presumably would have been assigned an acute effects assessment concentration (EC_A) of 18.93 ug CN/L. That brings up to 32 the number of fish species initially warranting an acute effects analysis.

None of the acute EC_As that fell below the current acute (CMC) criterion for cyanide were derived from directly measured exposure-response curves for acute exposures to cyanide among any of the 32 species of fish and 1 species of invertebrate listed above. All of the EC_As in question were estimated from eleven ICE (Interspecies Correlation Estimates) models matching eleven taxonomic groupings (such as *Etheostoma* darters) of the species listed above. EPA (2007) derived the BE EC_As by calculating lower 90% confidence limit values for ICE-estimated acute LC_{50}s and then dividing those surrogate LC_{50} estimates by a lethality threshold adjustment factor (LTAF) of 2.27 to adjust the expected effects level downward from 50% lethality to a level estimated to fall somewhere between 0-10% (EPA 2006).

The LTAF of 2.27 is based on a compilation of data (n=219) for an assortment of chemicals, effluent waters of unknown chemistry, and test species that was published by EPA in the May 18, 1978 *Federal Register* (43 FR 21506). In Section 3.3.1.1 of EPA's (2006) *Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria, Methods Manual* it is recommended, if possible, that the generic LTAF of 2.27 be reviewed for appropriateness when applied to particular chemicals and species of receptor organisms. Such a review was not part of EPA's (2007) final BE. However, it was noted in Gensemer et al. (2007) that a LTAF substantively lower than 2.27 appeared to be warranted based on response data for acute exposures of Rainbow Trout to aqueous cyanide.

<u>Review of 1978 LTAF data compilation for applicability to cyanide:</u> An examination of the 219 LTAFs published in 1978 (43 FR 21506) revealed that none of those data came from studies of cyanide acute toxicity. It also revealed that there was no standardization of the "threshold" effect level associated with the compiled LTAF values. The adjustment factors were believed to vary from LC_{50}/LC_{01} to LC_{50}/LC_{10} ratios. Due to such variable "threshold" reference points, along with the other sources of variability inherent in a universally pooled sample of multiple chemicals and multiple test organisms, the reported estimates of LTAFs ranged from as low as 1.10 to as high as 50. Clearly, applying the geometric mean (2.27) of such a broad range of candidate LTAFs introduces a substantive source of uncertainty into estimates of EC_As.

<u>Calculating EC_{10} standardized cyanide-specific LTAFs for fish:</u> Subsequent to EPA's (1978) *Federal Register* publication of the generic LTAF data compilation, cyanide-specific data for several species of fish and life stages were published by Smith et al. (1978) and Broderius and Smith (1979). Furthermore, these authors published acute exposure-response regression equations which provide a basis for calculating standardized LTAF estimates.

If data were statistically powerful enough to support it, LTAFs ideally should be standardized to an LC_{50}/LC_{01} ratio. The LC_{10} was chosen for the standardization point for two reasons: (1) it has previously been used as a point of standardization for toxicological work on ESA-listed species (Dwyer et al. 2005) because "*... a 10% level of mortality... is considered acceptable control mortality* [in typical toxicity testing experimental bioassays] and (2) because the specific 95% lower confidence boundaries for estimated chronic EC_{10}s from all the effects regressions are zero (unlike for the EC_{20}s; Appendix F) indicating that the regressions don't have the statistical power to allow standardization at a lower EC/LC level. Although, this biological opinion standardizes re-calculated LTAFs to the LC_{10} level of acute toxic response, whenever best available data can support a more statistically powerful estimate of toxic thresholds those alternatives would be preferred.

There are 62 acute exposure-response regression equations from which LC_{50}/LC_{10}-standardized estimates of LTAFs can be calculated (Appendix G?). Results of those calculations can be summarized as follows:

Life Stage	Species	Mean LC_{50}/LC_{10} LTAFs
Eggs / Sac Fry:	Fathead Minnow n=5	1.89
	Brook Trout n=4	2.09
	GM of spp. means	**1.99**
Fry:	Fathead Minnow n=5	1.55
	Bluegill n=4	2.09
	Brook Trout n=5	1.40
	GM of spp. means	**1.66**
Juvenile:	Fathead Minnow n=16	1.28
	Bluegill n=7	1.23
	Yellow Perch n=6	1.24
	Non-salmonid spp. GM	**1.25**
	Brook Trout n=9	1.15
	Rainbow Trout n=1	1.14
	Salmonid spp. GM	**1.14**
	Pooled Fish spp. GM	**1.21**

As reviewed by Eisler (2000), for fish the juvenile life stage is more sensitive to cyanide than the egg, sac fry or fry life stages. EPA's guidelines for deriving water quality criteria (Stephan et al. 1985) stipulate that they be derived from toxicity test data for the most sensitive life stage. Accordingly, the cyanide-specific, and LC_{50}/LC_{10}-standardized, LTAF results presented above for the juvenile life stage are the most applicable values for recalculating acute EC_As. Those values are substantively lower than the generic LTAF value of 2.27 from the 1978 *Federal Register (*43 FR 21506) data compilation.

Recalculated acute effects assessment concentrations (EC_As): In addition to recalculating acute EC_As based on revised LTAFs, the recalculations presented in Table B1 also adjust the ICE LC_{50}s from the lower 90% confidence values used in the final BE (EPA 2007) to the lower 95% confidence values as stipulated in the BE Methodology guidance document (EPA 2006). As indicated above by boldface type (Table B1), there are now only four species of fish, within FWS' jurisdiction, that have estimated acute EC_As lower than the acute (CMC) criterion of 22.4 ug CN/L. Those species include one darter (Fountain Darter) and three species of salmonids (Apache Trout, Bull Trout, and Lahonton Cutthroat Trout). Gensemer et al. (2007) also identified the Fountain Darter and Apache Trout as species that were likely not fully protected by the current acute (CMC) criterion for cyanide.

Appendix B2

Recalculation of the Acute to Chronic Ratio (ACR) for Fish

During the course of the effects analysis for this biological opinion it became evident that the original "likely to adversely affect" (LAA) screening was not "calibrated" properly because few species other than those associated with fairly high levels of predicted chronic effects (>35%) were screened onto the original list of LAA species. For detailed elaboration on this outcome see the section of this biological opinion titled, *"Derivation of the criterion continuous concentration (CCC)"*. It was apparent that the level of effect associated with NOECs was highly variable (unstandardized), sometimes quite high, and that use of NOECs to calculate ACRs, as was done by EPA in the Cyanide BE ($ACR=LC_{50}/NOEC$), resulted in screening values for listed species (i.e. Chronic Assessment Effects Concentrations or Chronic EC_A) that were based on unacceptably high levels of effect. As was stated earlier in Appendix B, the Chronic EC_A represents *the highest* concentration of cyanide where adverse effects on listed species are not likely, thus ensuring adequate protection for listed species exposed to cyanide at or below the Chronic EC_A. We developed an alternative approach for calculating ACRs, such that, the resulting Chronic EC_As are more appropriate for the LAA screening process.

The chronic effects regressions that were developed for this biological opinion provide a basis for calculating standardized ACRs, and thus provide a basis for a more scientifically rigorous and conceptually transparent LAA screening criterion. ACRs standardized to an EC_{10} were chosen for re-calibrating the LAA screening. The EC_{10} was chosen for the standardization point for two reasons: (1) it has previously been used as a point of standardization for toxicological work on ESA-listed species (Dwyer et al. 2005) because "*... a 10% level of mortality... is considered acceptable control mortality* [in typical toxicity testing experimental bioassays] and (2) because the specific 95% lower confidence boundaries for estimated EC_{10}s from all the effects regressions are zero (unlike for the EC_{20}s; Appendix F) indicating that the regressions don't have the statistical power to allow standardization at a lower EC level. Under ideal circumstances, ACRs would be standardized to an EC_{01} magnitude of chronic effects (i.e., standardized to a true threshold level of effect), but that simply cannot yet be supported by existing data.

Below are the re-calculated EC_{10} standardized ACRs for the chronic effects regression model species of fish:

Model Species	LC_{50} Values ug CN / L	EC_{10} Values ug CN / L	Standardized ACRs
Fathead Minnow	138.4	4.43	31.24
Brook Trout	85.7	2.64	32.46
Bluegill	126.1	4.61	27.35

Simply using the geometric mean of these three re-calculated and EC_{10} standardized ACRs (i.e., 30.27) for a re-calculated LAA screening ACR would not make use of all six species of fish for which unstandardized ACRs were listed in the BE (EPA 2007). A method to indirectly incorporate information from all those species is to compute ratios of EC_{10} standardized ACRs to BE ACRs (EPA 2207) for each of our three regression model species, then calculate the average of those three ratios to use as a multiplication factor for EC_{10} standardizing the BE geometric mean fish ACR (which EPA derived from all six species of fish for which unstandardized ACRs were available). Accordingly, the EC_{10} standardized ACR to BE ACR ratios were 2.41, 2.14, and 2.04 respectively for fathead minnow, brook trout, and bluegill. The average EC_{10} standardized to BE ACR ratio is therefore 2.197. Multiplying the BE geometric mean fish ACR of 10.57 by 2.197 yields a re-calculated geometric mean EC_{10} standardized ACR of 23.22. This is the ACR that was used to "re-calibrate" the LAA screen. This change moved the critical screening value, based on LC_{50}'s, from LC_{50}s <55 ug CN / L (for a species to be classified as LAA), up to an LC_{50} <121 ug CN / L.

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